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IMMUNOBLOT ANALYSIS OF ANTIBODY RESPONSE IN MICE INFECTED WITH *COXIELLA BURNETII* PHASE I

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Summary. - Mouse sera collected from day 4 to day 133 postinfection (p.i.) with phase I *Coxiella burnetii* strain Nine Mile were analysed by immunobloting with phase I *C. burnetii* cell lysate. Antibodies of IgG class protein antigens were revealed already on day 10 p.i. (60, 49 and 27 kD proteins), followed by those to 77 kD protein from day 18 p.i. and to further 7 (from 42 to 70 kD) proteins from day 28 p.i. IgG antibody reaction was observed also with 5 antigens in 14-20 kD region corresponding to lipopolysaccharides from day 22 p.i. Suprisingly, antibodies of IgM type appeared later (from day 22 p.i.) and were directed only to protein antigens, most markedly to 60 and 77 kD proteins. Differences in immunoblot patterns observed with the serum collected on day 72 p.i. before and after absorbtion to phase I and phase II *C. burnetii* cells, and to phase I cells treated by trichloroacetic acid (TCA) or KIO_4, indicate the surface localization of protein phase I antigenic epitopes, which can be destroyed partly by TCA and almost completely by KIO_4 treatment.

Key words: C. burnetii; antibody response; immunoblot analysis; experimental animals

Introduction

Unique properties of *Coxiella burnetii* differentiate this causative agent of Q fever from other pathogenic rickettsiae (Baca and Paretsky, 1983). They are probably based on the pecularities of *C. burnetii* surface structures, namely lipopolysaccharides (LPSs) and presumably also proteins, to which a great attention has been paid during the last decade (Schramek and Mayer, 1982; Williams and Stewart, 1984; Hackstadt *et al.*, 1985; Müller *et al.*, 1987; Hackstadt, 1988; Schmeer, 1988; Novák and Brezina, 1989).

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In our study the dynamics of antibody response to protein and LPS components of *C. burnetii* in mice infected with a phase I strain of this agent was analysed by immunoblotting to find out their relative immunogenic significance.

Materials and Methods

C. burnetii phase I Nine Mile strain (the 2nd chick embryo yolk sac passage) was grown in embryonated eggs, purified by Renografin density gradient centrifugation,standardized to a final concentration of 1 mg/ml in phosphate buffered saline, pH 7.2, and stored at -20 °C. This stock titrated in embryonated eggs was used to infect intraperitoneally SPF outbred mice of the VELAZ breed, weighing 18-20 g in a dose of 10^6 EID_{50}. On days 4, 7, 10, 14, 18, 22, 28, 52, 72, 100, and 133 post-infection (p.i.), groups of 6 mice were bled from the sinus orbitalis and the sera obtained were analysed by immunoblotting.

SDS-PAGE for immunoblotting was done using the discontinuous buffer system (Laemmli, 1970) with application of 10 μg of *C. burnetii* lysate per 1 mm of a 10-18 % (w/v) gradient of polyacrylamide gel. Immunoblotting (Western blot) was performed according to the procedures described elsewhere (Towbin *et al.*, 1979; Andersen *et al.*, 1987). Swine antimouse IgG-peroxidase conjugated (ÚSOL, Prague) and goat antimouse IgM-conjugated (heavy chain specific; Southern Biotechnology Associates, Inc., Birmingham, Alabama, U.S.A.) were used as secondary antibodies.

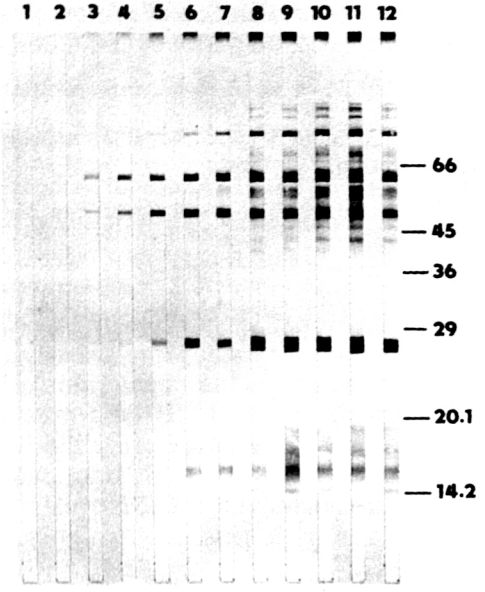

Fig. 1
Immunoblot analysis of antibodies of IgG class in mouse sera on days 4 - 133 p.i. (Nos. 1-12)

In last series of our experiments, the serum sample collected on day 72 p.i. was used for further immunoblot analysis after adsorption with 1 mg of killed purified phase I or phase II *C. burnetii* cells, and with phase I cells residues obtained by trichloroacetic acid (TCA; Brezina and Úrvölgyi, 1962) and potassium periodate (Schramek *et al.*, 1972) treatment. Both non-adsorbed (supernatants resulting from 3 adsorbtion cycles for 30 min at 37 °C each, followed by 5 min centrifugation at 14,000 x g) and adsorbed but re-obtained (by 30 min incubation at room temperature of remaining *C. burnetii* pellets with 0.2 mol/l glycin-HCl, pH 2.8, and 0.15 mol/l NaCl followed by further centrifugation) fractions were used and compared in the immunoblotting with a phase I *C. burnetii* cell lysate.

Results and Discussion

The dynamics of IgG class antibody spectrum development to different protein and LPS components of phase I *C. burnetii* cells is shown in Fig. 1. The first antibodies were detected to 60, 49, and 27 kD proteins already on day 10 p.i. with the maximum on days 22–100 p.i., followed by antibodies to 77 kD protein from day 18 p.i. and to further 7 (from 42 to 70 kD) proteins from day 28 p.i. Sera reacted also with 5 antigens of different molecular masses in the lower region of 14–20 kD, corresponding to LPSs, from day 22 p.i. with the maximum on day 52 p.i.

Fig. 2 presents the results of immunoblotting specific for antibodies of IgM class. Distinct lanes were obtained with proteins only, namely those of higher

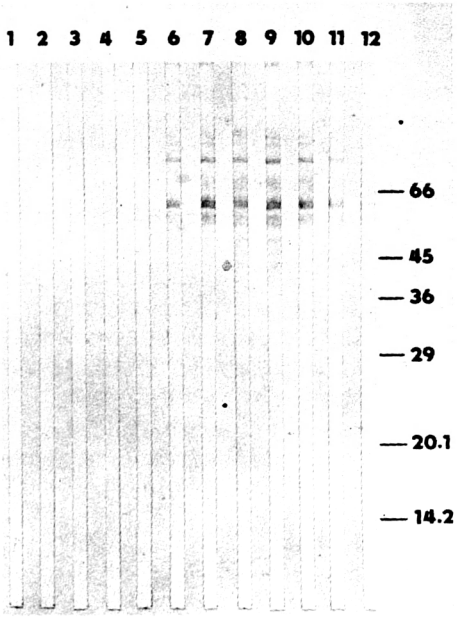

Fig. 2
Immunoblot analysis of antibodies of IgM class in mouse sera on day 4 - 133 p.i. (Nos. 1-12)

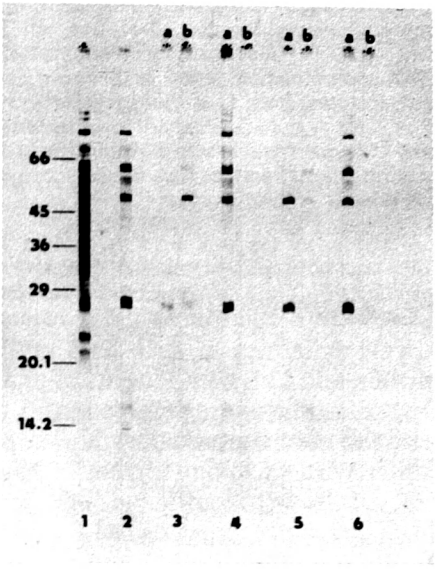

Fig. 3
Immunoblot analysis of immune mouse sera (day 72 p.i.) intact (lane 2), bound to and eluted from phase I (lanes 3a and 3b) and phase II (lanes 4a and 4b) cells, and phase I cells treated with TCA (lanes 5a and 5b) or KIO_4 (lanes 6a and 6b); lane 1 indicates markers of molecular mass

molecular mass, the most marked with 60 and 77 kD proteins. They also appeared later (day 22 p.i.) than lanes specific for antibodies of IgG type (days 10 and 18 p.i.) and disappeared on the last day of observation (133 p.i.) If this were true, the kinetics of IgM and IgG *C. burnetii* specific antibody response should have differed from that known from previous studies (Fiset and Ormsbeee, 1968; Kazár *et al.*, 1977). This finding deserves further attention, though the possibility of lower detectability of antibody response of IgM than IgG class in our study is not excluded.

Of interest is an observation found with the fractions of serum (collected on day 72 p.i. at the peak of antibody response as demonstrated by immunoblotting) adsorbed and non-adsorbed to phase II *C. burnetii* cells and untreated, TCA-or KIO_4-treated phase I *C. burnetii* cells (Fig. 3). As follows from comparison of sheets 3a and 4a with the sheet 2, the intact mouse p.i. serum contained antibodies which almost completely bind to the surface antigenic structures of phase I but not phase II cells. This finding is supported by the elution of antibodies bound to the phase I (sheet 3b) but not phase II (sheet 4b) cells. Adsorbtion of serum ot TCA-treated phase I cells led to partial disappearance of some lanes after antibody binding (sheet 5a) and reappearance of other lanes after antibody elution (sheet 5b). On the other hand, adsorbtion of the serum to KIO_4-treated phase I cells resulted in the pattern similar to that observed after serum adsorbtion to phase II cells (see sheets 6 and 4). From this follows the surface localization of antigenic epitopes of phase I *C. burnetii* cells, which can be easily destroyed or removed by KIO_4 than by TCA treatment.

The immunogenicity of protein and/or LPS epitopes of *C. burnetii* remains to be elucidated, though some authors suggested the importance of 27 kD protein (Müller *et al.*, 1987; Schmeer, 1988) and 29.5 kD protein (Williams *et al.*, 1990). We are of the opinion that apart from these proteins of lower molecular also proteins of higher molecular mass should be considered as potential immunogens, when taking into account that 60–65 kD proteins were found common for bacteria (Young *et al.*, 1987; Picketts *et al.*, 1989) including the homology of major *C. burnetii* 62 kD protein with that of *Mycobacteria* and *Escherichia coli* (Vodkin and Williams, 1988). However, to determine relative importance of individual proteins and/or LPS surface epitopes of *C. burnetii* in protection from Q fever, further studies using monoclonal antibodies are necessary.

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